

FINAL REPORT

Project Title: Development of Gravity Sensitive Plant Cells (Ceratodon) in Microgravity

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Monitoring Center: ARC

Grant Number: ~~NAG2-1217~~

NASA Solicitation: 96-OLMSA-01

Total Funding: \$34,247

Start Date: May 1, 1998

End Date: August 31, 1999

Joint Agency:

Flight Assignment: STS107 (pending)

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Number of Funded Students:

Pre-college: **Undergraduate:** 3 **Graduate:** **Post-doctoral:** 1

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INTRODUCTION

Protonemata of the moss *Ceratodon* are tip-growing cells that grow up in the dark. This cell type is unique compared to cells in almost any other organism, since the growth of the plant cell itself is completely oriented by gravity. Thus, both the processes of gravity sensing and the gravity response occur in the same cell. Gravity sensing appears to rely upon amyloplasts (starch-filled plastids) that sediment. This sedimentation occurs in specific zones and plastid zonation is complex with respect to plastid morphology, distribution, and gravity. Microtubules restrict the extent of plastid sedimentation (i.e., they are load-bearing). Light also is important since apical cells have a phytochrome-based positive phototropism, light quality influences plastid zonation and sedimentation (photomorphogenesis), and red light suppresses gravitropism at higher but not lower light intensities.

Many of these processes were examined in a 16 day spaceflight experiment, SPM-A ("space moss" or "SPAM") on STS-87 that landed in December, 1997. The work described here involves the definition of a second flight experiment that builds upon the data and questions arising from STS-87.

Effort was directed towards further definition of an experiment for the Shuttle (dubbed "SOS" for "Son of SPAM"). Our current target is STS 107 that is scheduled to fly in January 2001. This definition addressed two goals of the STS107 experiment.

CONTEXT AND DEFINITION

Goal 1: Determine whether the cytoskeleton plays a role in maintaining and generating an apical (non-random) plastid distribution in microgravity

Context: Goal 1

Since amyloplasts sediment along the length of apical cells in upright and inverted positions, the prediction for microgravity was that the amyloplasts that normally sediment would be randomly distributed without a g cue. Instead in SPM-A, dark-grown cells had amyloplasts that were concentrated in apical clusters. This distribution is similar, but not identical to cells that have been inverted or rotated on a clinostat at 1-g. Thus it is striking that in microgravity, amyloplasts like the tips of the cells, were distributed non-randomly.

In SOS we plan to expand the sample of cells examined to extend previous observations on amyloplast distribution. The finding of an apical cluster is based on a sample of about 40 apical cells grown in microgravity that were photographed shortly after landing. Moreover, although almost all cells show an apical distribution of plastids in microgravity, there is considerable variability cell to cell in the details of plastid distribution. Obviously an expanded sample as well as a second experiment are important to rigorously confirm the non-random distribution. Also, our analysis of the SPM-A results suggests that there are subtle visual and quantitative differences between cells exposed to microgravity and those rotated on a clinostat. To determine conclusively that these differences are real, additional samples are needed.

Another major focus of SOS will be to explore whether the cytoskeleton participates in maintaining and generating the apical cluster in microgravity. We have previously shown (Cell Motility and Cytoskeleton 29: 366-74) that sedimentation at 1-g

is normally restricted by microtubules, not microfilaments. Because microtubules may be load bearing for amyloplasts, it is possible that when this load is released in microgravity that microtubules function in positioning the amyloplasts close to the cell tip. (Two hypothetical mechanisms are that a g-induced strain normally controls the degree of tubulin polymerization and that there is a default organelle motility that is normally counteracted by gravity).

To ascertain whether the cytoskeleton is involved in amyloplast positioning in microgravity in SOS, cytoskeletal inhibitors (oryzalin and cytochalasin which depolymerize microtubules and microfilaments respectively) will be applied in microgravity for 1, 6 and 24 hours after which the cultures will be fixed. Before inhibitor treatment, plastids should already be apically distributed since the cells have been growing in microgravity. Thus, in the earlier time points, we will be testing whether cytoskeletal integrity is necessary for maintaining the apical cluster. It is possible, for example, that the application of the inhibitors will cause the randomization of amyloplast position which would implicate the cytoskeleton.

The 24-h application of oryzalin offers a test of whether the microtubules are necessary for the formation of the sub-apical cluster *de novo*. In ground-based experiments, we have found that oryzalin causes the normally cylindrical cells to bulge at the tip and then eventually new tips emerge from the bulge which are cylindrical again and which often exhibit normal plastid zonation. If the tips that regenerate after prolonged oryzalin treatment in microgravity lack an apical cluster, then this would suggest that microtubules are necessary for the generation of the non-random distribution. Alternatively, it could indicate that the microtubules somehow adapt to the presence of the inhibitor. Some of the treated and untreated material will be embedded post-flight in media suitable for characterizing the organization of the cytoskeleton - especially microtubules - by immunofluorescence.

The finding that amyloplasts in microgravity-grown cells are distributed close to the apex suggests that endogenous forces act constitutively on these organelles. Gravity can counteract these forces as evidenced by amyloplast sedimentation in upright cells on the ground. Because microtubules are load-bearing for amyloplasts at 1-g, it is logical that they might also be important for the positioning seen in microgravity. While more analysis is needed to understand the mechanism, these data support the idea that both intrinsic and extrinsic forces control the position of dense organelles. These results are also consistent with the hypothesis that the cytoskeleton evolved, at least in part, to prevent the stratification of organelles only by density.

Definition: Goal 1

A major goal was to define the parameters for testing the role of the cytoskeleton in positioning plastids in microgravity. To this end we determined, in conjunction with engineers at Kennedy Space Center, how to adapt existing hardware (the Petri Dish Fixation Unit or PDFU) to accommodate the applications of cytoskeletal inhibitors before chemical fixation. This involved testing the efficacy of different concentrations, volumes and exposure periods of inhibitors and fixatives.

Goal 2: Describe the development and extent of clockwise spiral tip-growth in microgravity

Context: Goal 2

One major goal of SPM-A was to determine whether the growth of these cells was random in microgravity. Since the cells grow straight up in the dark at 1-g, one prediction for flight was that orientation would be random, just as in roots and shoots in space. However, cultures showed clear clockwise spirals in STS-87. In the three cultures grown in the dark for 7 days in flight, spirals were not distinct. But in the three cultures grown in the dark for 14 days, the spirals were clear. This suggests that cultures need to reach a certain age or stage of development before the spirals are expressed.

In a separate treatment in our CUE experiment, we exposed cells to unilateral red light for 7 days and then put them into darkness for 7 days. After 7 days of unilateral red light, most cells grow towards the light, but some grow directly away (positive and negative phototropism respectively). Thus after 7 days in the light the culture was essentially aligned in a straight line in the light path. After 7 days in the dark, the tips formed clockwise hooks at each end. This suggests that 7 days in microgravity is sufficient to induce clockwise spirals as long as the culture is at least a week old before exposure to microgravity. However, the SPM-A experiment did not study the effects of more than 7 days or less than 7 days in the dark following red light pre-treatment.

As far as we are aware, the SPM-A results represent only the second time that any gravitropic plant cell or organ grew non-randomly in space (cress roots form arcs related to the seed that produces them). One objective of SOS is to confirm the important result that spiral growth is a predictable response to flight. A second is to determine the age at which clockwise growth starts to be expressed in the dark in material that has been grown entirely in the dark compared to material given a pre-orienting red light treatment. Entirely dark-grown material will be fixed at 7, 10.5 and 14 days, and red-light pretreated cultures will be fixed at 3.5, 7, 10.5 and 14 days. The latter treatments will tell us if the clockwise hooks form earlier than 7 days and whether these hooks keep curving clockwise over a longer-term exposure to microgravity.

The presence of coordinated clockwise spiral growth in microgravity suggests that there is an endogenous growth polarity in *Ceratodon* that normally is suppressed by gravitropism. A working hypothesis is that the spirals represent a residual spacing mechanism for controlling colony growth and the distribution of branches under some conditions and in some mosses. But considerable further study is needed to put these unusual findings in context.

Definition: Goal 2

Additional tests were necessary to assess the consequences of different lengths of Shuttle flights and of pre-flight loading. These concerns were mainly directed towards the development of spiral growth.

We also explored pre-treatments that can increase the number of times points in microgravity. Cultures loaded into the Shuttle would be given a red-light pre-treatment on the ground. The light would then be turned off in orbit. This would nullify the g effects of loading the dishes in the Shuttle for 1-2 days in an incorrect orientation. It would also obviate g effects of the launch. This is because moderate intensity red light

phototropism is much stronger than gravitropism. We tested several schedule combinations of days of unilateral red light followed by varying the days in the dark on a clinostat. We conclude that a red-light pre-treatment will allow us to use the full duration of microgravity by launching cultures that will be relatively mature.

Earth Benefits

This research primarily addresses fundamental questions in gravitational biology. One thrust is to shed light on how gravity controls the organization of cells. The flight experiment will help in understanding how the components of cells are regulated so as to not be simply stratified with respect to gravity based on their densities.

Another thrust is to understand how cell growth which is localized to one pole of a cell is regulated by the environment, i.e., how tip growth is modulated. Plant productivity depends in a major way on tip-growth for root growth and for fertilization and fruit and seed production. Knowledge about the regulation of tip-growth would thus be helpful for understanding one of the bases of agronomic yield.

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